

## **A Theoretical Mode of Action of Aldosterone†**

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Aldosterone is the most important of known sodium-regulating hormones, although it is responsible for only a few per cent of total renal sodium reabsorption. In addition, aldosterone can increase renal excretion of hydrogen and potassium ions. That its renal effects are direct was first demonstrated by Barger, Berlin & Tulenko (1958) by unilateral renal artery injection of the hormone into normal and adrenalectomized dogs. Moreover, these investigators were able to demonstrate a complete dissociation of aldosterone's kaluretic and anti-natriuretic effects in the normal dog. Ganong & Mulrow (1958), using a similar technic, have also found a significant separation of these effects in the adrenalectomized dog. Thus the possibility that aldosterone accelerates transport systems exchanging sodium for hydrogen and potassium remains an unresolved question.

Recently (Vander *et al.*, 1958; 1960), with the use of stop flow analysis technic (Malvin, Wilde & Sullivan, 1958), a distal site of action of aldosterone has been demonstrated. It is the purpose of this paper to describe a model for sodium transport and aldosterone activity based upon these findings.

### **Site of Action of Aldosterone**

By administration of an osmotic diuretic (mannitol) to the dog, very high rates of urine flow can be established. If during this period the ureter is clamped, intratubular pressure rises to equal net filtration pressure, at which point glomerular filtration ceases. During this period of stopped ureteral flow, the concentration of any substance in the intratubular fluid column will be changed along the nephron, depending upon how the individual segments handle this substance. The mannitol retards water reabsorption and provides a menstruum against which electrolyte concentrations may be changed. Upon release of occlusion, these concentra-

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tion patterns are obtained in approximately 0.5 ml urine samples which segment this pattern into an ordered array, best pictured on a graph if concentration of each sample is plotted against accumulative urine volume. Thus the dashed curve in Fig. 1 is a typical normal sodium concentration pattern, demonstrating a fall in sodium concentration as fluid trapped in the distal tubules during occlusion enters the urine collector after release of occlusion. The line designating PAH maximum indicates the collection of fluid trapped primarily in the proximal tubules during occlusion.

It is evident from Fig. 1 that, after adrenalectomy, the distal tubule was not able to reduce sodium concentration to the minimum value achieved during ureteral occlusion in normal dogs. The final concentration achieved was not altered by prolonging the length of occlusion.

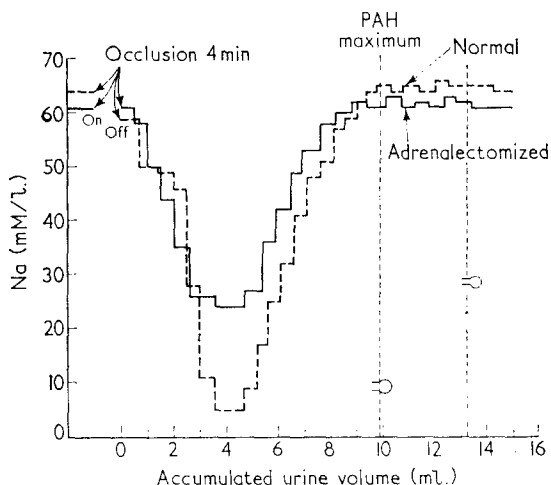


FIG. 1. Comparison of distal tubular sodium reabsorption during ureteral occlusion in a normal and in an adrenalectomized dog (Vander *et al.*, 1958: by courtesy of the Society for Experimental Biology and Medicine).

Also of significance was the finding (Fig. 2) that the minimum distal stop flow sodium concentration was a function of the plasma sodium concentration in the adrenalectomized but not the normal dogs. In the former group, as plasma sodium rose (this was effected by injection of hypertonic NaCl between occlusions), the minimal sodium concentration attained during ureteral occlusion also rose. This indicates that adrenalectomy has reduced the maximal sodium concentration gradient which could be maintained across distal tubular cells. Identical results were obtained by administration of the steroidal antagonist SC-8109 to normal dogs. Finally, all the above abnormalities could be corrected by the administration of aldosterone.

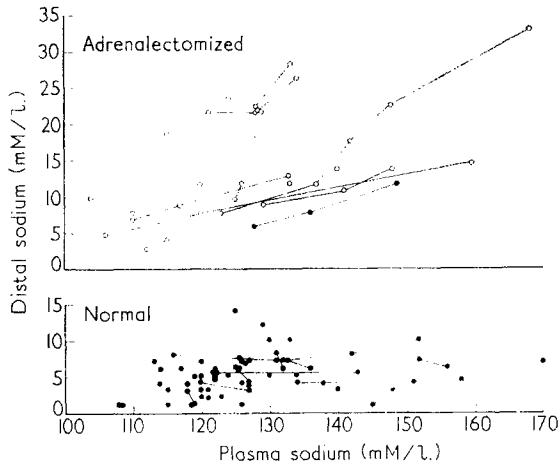


FIG. 2. Relationship between plasma sodium concentration and the minimal distal urinary sodium concentration developed during ureteral occlusion in normal and adrenalectomized dogs. Lines connect points derived from a single dog as plasma sodium was elevated (Vander *et al.*, 1960; by courtesy of the Society for Experimental Biology and Medicine).

### Mode of Action of Aldosterone

Conventional clearance methods indicate that aldosterone increases tubular reabsorption of sodium. This action is manifested in stop flow analysis by the ability of aldosterone to increase the maximal sodium concentration gradient which can be developed between plasma and distal tubular urine. Since the minimal distal sodium concentration attained during ureteral occlusion is independent of the duration of occlusion, this concentration must be an equilibrium value, i.e. the concentration at which sodium movement out of the lumen is equal to sodium movement inward. Aldosterone could act, therefore, in one of two different ways: it could activate carrier systems responsible for sodium transport outward or it might decrease passive back-diffusion of sodium from interstitial fluid into distal tubular lumen.

A rough estimate of such back-diffusion of Na is being made in our laboratory, using the isotope  $^{24}\text{Na}$ . The ureteral occlusion stops filtration so that glomerular substances such as inulin will not enter the concentration pattern except as new filtrate.  $^{24}\text{Na}$  injected intravenously after the occlusion, by crossing the tubular epithelium transmurally, enters the urinary pattern ahead of inulin. Precise delineation of rates of movement into the stop flow pattern is complicated by the continuing decline of the precursor  $^{24}\text{Na}$  in the blood plasma during the short two-minute period allowed for the  $^{24}\text{Na}$  to enter the tubule lumen. The shape of the stop flow

curves for  $^{24}\text{Na}$  seems unchanged after adrenalectomy or SC-9109 with no suggestion of any increased rate of back-diffusion.

### Effect on Kinetics of Active Transport

As mentioned above, the length of time of occlusion beyond 3 to 4 minutes does not alter the final sodium concentration achieved by the distal tubule during occlusion, indicating that this concentration represents an equilibrium value at which movement of sodium outward is equal to its movement inward. This fact allows us to develop the following equations, which can be used to characterize, subject to the limitations described below, not only the carrier systems responsible for active sodium transport but also the influence aldosterone exerts upon them.

$$D_{\text{in}} = K \text{Na}_p \tag{1}$$

$$D_{\text{out}} = K \text{Na}_i \tag{2}$$

where  $D$  = rate of passive diffusion of sodium inward or outward;  $K$  = diffusion constant for Na through distal tubular cells in either direction;  $\text{Na}_p$  = plasma sodium concentration;  $\text{Na}_i$  = minimum distal tubular sodium concentration attained during ureteral occlusion.

It must be pointed out, however, that the rate of these passive fluxes will be determined not only by the diffusion constant but also by the transtubular electrical potential gradient. Since this electrical gradient will oppose sodium flux in one direction and favor it in the opposite, depending upon the orientation of the charge, it cannot be incorporated into the parameter ( $K$ ). Giebisch (1958) using the technique of micropuncture, has made four determinations (27, 37, 39, 40 mV, inside negative to outside) of distal transtubular potential in *Necturus*. Interpolation of these data to the dog under conditions of stop flow would be unwarranted. Furthermore, since the origin of the potential is unknown, it is not possible to predict the effects of increasing plasma sodium concentration upon it. Because of the impossibility, at present, of incorporating the electrical potential into the equations below, the authors believe that it would still be of value to attempt to set up a working hypothesis, ignoring this factor, and subject always to question and modification.

If  $r_{\text{Na}}$  = rate of active reabsorption of sodium, then at equilibrium,

$$K \text{Na}_p = K \text{Na}_i + r_{\text{Na}}$$

$$K(\text{Na}_p - \text{Na}_i) = r_{\text{Na}} \tag{3}$$

The rate,  $r_{\text{Na}}$ , may be described by an equation analogous to the Michaelis-Menten equation of enzyme kinetics:

$$r_{\text{Na}} = \frac{R_{\text{Na}} \text{Na}_i}{\text{Na}_i + K_M} \tag{4}$$

Where  $R_{Na}$  = maximal or saturation rate of distal tubular sodium re-absorption;  $Na_i$  is as above;  $K_M$  = that sodium concentration in the distal lumen at which the velocity of active outward sodium transport, in analogy to the Michaelis-Menten constant of enzyme kinetics, is equal to  $\frac{1}{2}$  the maximal attainable velocity. Combining equations (3) and (4):

$$K(Na_p - Na_i) = \frac{R_{Na} Na_i}{Na_i + K_M} \quad (5)$$

In this equation,  $K$ ,  $R_{Na}$ , and  $K_M$  are all unknown. However,  $Na_i$  and  $Na_p$  can be determined for two different steady state concentration gradients, in two separate occlusions on the same dog, in one of which  $Na_p$  and  $Na_i$  are altered by elevating  $Na_p$ , resulting in an elevation of  $Na_i$ . These values can then be substituted separately into equation (5) to yield two equations. Divide one equation by the other:

$$\frac{K(Na_p^a - Na_i^a)}{K(Na_p^b - Na_i^b)} = \frac{R_{Na} Na_i^a}{Na_i^a + K_M} \bigg/ \frac{R_{Na} Na_i^b}{Na_i^b + K_M} \quad (6)$$

in which the superscripts  $a$  and  $b$  designate sodium concentrations at two different equilibria in the same animal. Since it is necessary that  $Na_i$  vary with  $Na_p$ , such an equation can be obtained only in experiments on adrenalectomized or SC-8109 treated dogs. In the normal animal, this cannot be done, distal sodium concentrations being essentially unchanged

TABLE I  
*Values of  $K_M$  in adrenalectomized and SC-8109 treated dogs*

$K_M$ mmoles/l	
SC-8109	Adrenalectomized
5.3	4.5
4.6	2.7
3.3	2.5
	1.7
	1.6

See text for definition of  $K_M$ .

at all plasma levels of sodium (very small changes, which very likely did occur, cannot be detected by stop flow analysis). The constants  $K$  and  $R_{Na}$  will cancel out and the equation may then be solved for  $K_M$ :

$$\frac{Na_p^a - Na_i^a}{Na_p^b - Na_i^b} = \frac{Na_i^a(Na_i^b + K_M)}{Na_i^b(Na_i^a + K_M)} \quad (7)$$

Table I lists values of  $K_M$  calculated for adrenalectomized and SC-8109 treated dogs. Evidence to be presented below indicates that  $K_M$  is identical

in intact animals. It can be seen that  $K_M$  is relatively constant in different animals and is quite low. This indicates that distal sodium reabsorptive systems operate at near maximal velocity over a wide range of intratubular sodium concentrations.

The determination of  $K_M$ , as described above, depends upon the basic assumption that the Michaelis-Menten equations developed do actually apply under the existing experimental conditions. However, it is not at first clear how these might be tested. Absolute velocities cannot be determined,

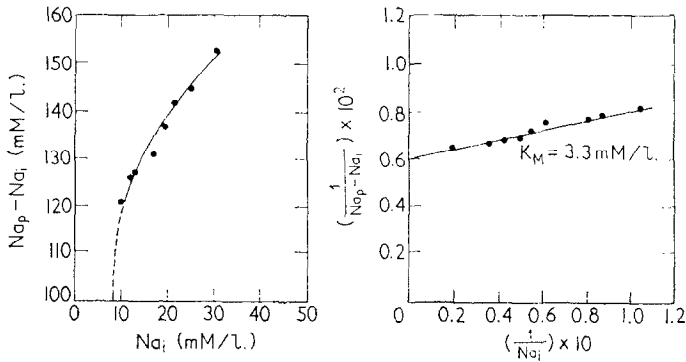


FIG. 3. "Velocity-substrate" plot of data obtained in an SC-8109 treated dog by elevating plasma Na.  $Na_p$  = plasma Na;  $Na_i$  = distal urinary Na concentration developed during ureteral occlusion.  $(Na_p - Na_i)$  is the proportional velocity of Na reabsorption. See text for discussion of  $K_M$ .

nor can they be calculated since a value for the diffusion constant,  $K$ , in equation (3) is unknown. Although it would be interesting to evaluate  $K$ , the validity of fitting the data to these equations can be determined by another device. This fact can be appreciated by referring back to equation (3). It is important to note again that this equation applies only at equilibrium. It is evident that for any given sodium concentrations in plasma and distal tubular fluid, velocity of sodium reabsorption will be proportional to the difference between the concentrations  $(Na_p - Na_i)$ . If the difference were to be increased by a factor of two, then the relative equilibrium velocity would also be doubled. It becomes evident, therefore, that these concentration differences can be used to represent transport velocities at each of the distal tubular sodium concentrations obtained within the same animal. Different dogs can be compared in this manner only if it is assumed that the diffusion constant,  $K$ , is identical in all animals.

Data obtained for an SC-8109 treated animal in which distal tubular sodium concentration achieved during stop flow was changed by increasing plasma sodium concentration are shown as a reciprocal plot in Fig. 3.

These "velocity-substrate" plots indicate that the data really fit a Michaelis-Menten type analysis. The intercept on the vertical axis in Fig. 3 is equal to the reciprocal of the maximal attainable transport velocity,  $1/R_{Na}$ . If the line is extended to the left, the intercept on the horizontal axis is equal to  $-1/K_M$ , thus providing a graphical method by which  $K_M$  can be determined. It is evident that this plot of reciprocals is merely a repetition of the use of equation (7) and will yield the same values for  $K_M$ , but only if the data really are linear, i.e. fit the proposed analysis. All values for  $K_M$  (see Table 1) derived by equation (7) are identical to those determined independently by this graphical means. The fact that the data are linear may further indicate that the possible error introduced by omitting electrical potentials<sup>2</sup> may not significantly alter results.

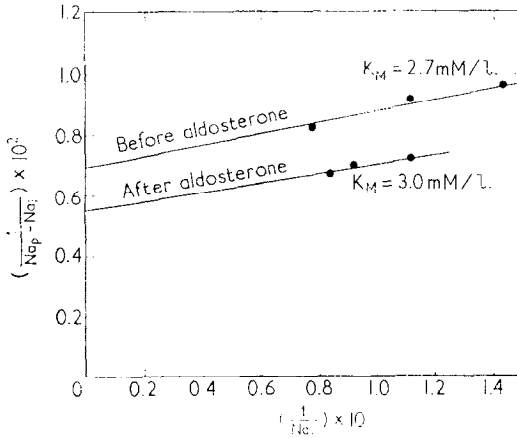


FIG. 4. Effect of aldosterone on carrier systems responsible for distal sodium reabsorption in an adrenalectomized dog.  $Na_p$  = plasma Na;  $Na_i$  = distal urinary Na concentration developed during ureteral occlusion.  $(Na_p - Na_i)$  is the proportional velocity of sodium reabsorption. See text for discussion of  $K_M$ .

Figure 4 demonstrates the action of aldosterone upon distal sodium reabsorptive systems, as revealed by these graphical methods. Three occlusions were performed in an adrenalectomized dog, increasing plasma sodium after each occlusion. After completion of the third occlusion, 6  $\mu$ g aldosterone were administered intravenously over 5 minutes and 1  $\mu$ g/hr was incorporated into the normal infusion. Three occlusions were then performed at 15 minute intervals after first waiting 2 hr 10 min. Plasma sodium was again elevated by salt injection between occlusions. Aldosterone dosage was kept at this level so that it permitted a degree of sodium elevation in the distal tubule as plasma sodium was elevated. As already described, this is extremely difficult to do in a normal animal or in an

adrenalectomized animal given large amounts of aldosterone. It should be pointed out that comparison depends upon the assumption that the diffusion constant,  $K$ , is not changed by aldosterone, an assumption believed to be valid on the basis of the isotope studies described earlier.

From the data shown in Fig. 4, values for  $K_M$ , before and after aldosterone, can either be calculated using equations described above, or graphically determined by extending the lines to the left and measuring the intercepts on the horizontal axis  $-1/K_M$ . It is this constant, a measure of the degree of association between carrier and sodium (in analogy to the affinity of an enzyme for its substrate) that would be altered by a competitive inhibitor or a coupling activator, but it can be seen that  $K_M$  is essentially unchanged by aldosterone.

Without changing this intercept on the horizontal axis, aldosterone has decreased the intercept on the vertical axis  $1/R_{Na}$ , so that the entire "velocity-substrate" line lies under that determined for the untreated adrenalectomized dog. Thus, aldosterone, without altering  $K_M$ , has increased transport velocity at each substrate concentration as well as having increased the maximal transport velocity attainable at infinite substrate concentration. This is the pattern proposed for a "non-coupling" activator (Friedenwald & Maengwyn-Davies, 1954). Stated more simply, aldosterone does not change the characteristics of the carrier systems, but increases the effective concentration of these carriers available for sodium reabsorption.

Although the data fit the Michaelis-Menten equation, this does not exclude the possibility that some other type of transport system geared to the saturation principle is involved. However, since there is no direct evidence bearing on the exact nature of the Na transport system, one can only speculate as to possible reactions involved. The above analysis merely presents a model for Na transport and describes the influence of aldosterone upon that system.

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